

REMARKS

The Interview

Applicant would like to thank Examiner Lankford for the courtesy of a personal Interview on December 13, 1995. At the Interview, Examiner Lankford indicated that claims reciting *Pythium insidiosum* as the source of fungal oil appeared to be allowable. Examiner Lankford stated that claims to use of fungal oils containing at least 10% ARA as ingredients would appear to be free of the prior art, including, for instance, claims reciting methods of using these oils as ingredients to make infant formula. Applicant pointed to claims directed to methods of making infant formula which were already in the application, and further pointed out that infant formula products (as currently claimed) included a requirement that ARA be provided as a fungal oil. Applicant explained that an oil-containing product in which ARA is provided by fungal oil would be inherently different from a similar product containing the same amount of ARA, but provided as fish oil, egg yolk, or free fatty acids. Examiner Lankford agreed that claims to products containing fungal oil in which ARA was present as a triglyceride appeared to be allowable.

However, with regard to claims directed to fungal oil *per se*, Examiner Lankford indicated that he would like further comments in the record concerning claims to fungal oils generally and claims to methods involving oil production using *M. alpina*. Applicant stated that he intended to cancel claims to a fungal oil containing 10% ARA as triglyceride, while retaining claims to fungal triglyceride oil containing 30% ARA. Detailed analysis of the art disclosed in the Information Disclosure Statement indicates that fungal oils containing at

least 30% ARA in triglyceride form could not have been described in this art (based on the data disclosed therein).

Examiner Lankford agreed to consider claims to fungal oil with 30% ARA in triglyceride form, and such claims are retained in the application subsequent to the present amendment. Examiner Lankford also agreed to addition of claims to fungal oil containing at least 50% ARA in triglyceride form, based on examples in the present application. Examiner Lankford pointed out that the term “unmodified” as part of the recitation of fungal oil in the claims was unnecessary to patentability. Therefore, he suggested that the term “unmodified” be deleted from the oil claims to eschew superfluous claim language.

The Amendments

Claims 19-21, 24, 30-32, 34, 35, 37, 41, 42, 44 and 50 are amended herein by deleting the word “unmodified” before the words “fungal oil”, as the word “unmodified” was indicated to be unnecessary by Examiner Lankford during the Interview. Claims 19 and 20 are amended herein to recite that the claimed fungal oil is an oil that has at least 30% ARA in triglyceride form. Such an oil was recited in original claim 21, and throughout the specification, *inter alia*, on pages 18-20. Further, the oils described on pages 19-20, and particularly those exemplified in Tables 5 and 6 (pages 36-37), contain at least 40% ARA (see, e.g., Table 5: log hours 94 and greater; and Table 6: log hours 90 and greater) or even 50% or greater (see, e.g., Table 5, log hours 237 and Table 6, log hours 139 or greater). Therefore, claim 21 is amended herein to recite that the claimed fungal oil is a triglyceride oil that has at least 40% ARA, and new claims 53-55 are added reciting fungal triglyceride oil having at least 50% ARA.

In addition, Applicants have noticed that claim 22 is a multiple dependent claim depending from another multiple dependent claim, and therefore the claims have been amended to change dependency of claim 22 without changing its scope. Specifically, claim 22 has been amended to avoid dependency from multiple dependent claim 21, and new claims 51 and 52 (which are singly dependent from multiple dependent claim 21) have been added. The above amendments being fully supported in the application as originally filed, do not add new matter to the application. Applicant respectfully requests that the above amendments be entered in the subject application.

The Invention

The present inventor has for the first time discovered how to obtain oil compositions having the desired amounts of unsaturated fatty acids using *microbial* oils which are high in arachidonic acid residues (ARA) and at the same time substantially free of eicosapentaenoic acid residues (EPA). Using one or more of these low EPA microbial oils, oil blends can be prepared having fatty acid ratios of polyunsaturated fatty acids (PUFAs) comparable to those in human breast milk, with the PUFAs in triglyceride form. Prior to the present invention, oil blends with this fatty acid composition could only be made by hydrolyzing plant or animal oils, purifying the resultant free fatty acids, and mixing the free fatty acids in the desired ratio.

The present inventor first recognized that fungal oils were valuable because triglycerides containing the desired fatty acid residues in the appropriate ratios can be readily obtained by extraction from the microbial source without need for further fractionation. In contrast, the desired ratio of fatty acid residues could not be obtained prior to the present

invention without, e.g., hydrolysis of ARA-containing oil under harsh conditions followed by fractionation of individual fatty acids. Such treatment routinely results in generation of undesirable side products which are absent from the fungal oils. The present claims are drawn to fungal triglyceride oils, methods of producing the oils and products made from the oils, and all require that the level of EPA be reduced relative to the ARA level and that the oils contain ARA in triglyceride form.

Rejection Under 35 U.S.C. § 103

Claims 1-50 stand rejected under 35 U.S.C. § 103 as unpatentable over two Japanese patent publications: JP 01/196,255, and JP 01/215,245. This rejection is respectfully traversed.

Claims to fungal oil from *Pythium insidiosum*, methods of producing this oil, products containing this oil and methods of making the products are patentable over the cited Japanese patent publications for the reasons set forth in the Information Disclosure Statement accompanying Applicant's Petition for Grant of Special Status filed July 3, 1995, as recognized by the Examiner at the Interview on December 13, 1995.

Prior to Applicant's priority document, there was no disclosure that any fungal oil could be prepared having at least 10% ARA in triglyceride form. When no oil with these characteristics was known, it could not have been obvious to use an unknown oil to prepare products containing such an oil. Therefore, the present claims to products containing fungal triglyceride oil having at least 10% ARA residues and to methods of making these products would not have been obvious over any prior art documents, and certainly not over the cited Japanese patent publications.

The highest level of ARA in any fungal biomass known to Applicant was reported by the Suntory group, who are also the inventors of the cited Japanese patent publications. In most of the publications by this group, the only measurements of ARA content reported are total ARA content in biomass or lipid extract. In a series of papers, Shinmen, et al., reported studies on lipid metabolism of *Mortierella* sp. Various strains of *Mortierella* were grown and the lipids were analyzed for ARA (and sometimes EPA) as a percent of total fatty acid content (see Shinmen, et al. (1989), *Appl. Microb. Biotech.*, 31:11-16; U.S. Pat. No. 4,916,066; U.S. Pat. No. 5,204,250; EP 0 223 960; and EP 0 276 982; Yamada, et al. (1987), Proc. World Congr. Biotechnol. for Oils and Fats Industr., Applewhite, ed., Am. Oil Chem. Soc., p. 173; Shimizu, et al. (1988), *J. Am. Oil Chem. Soc.*, 65:1455; Shimizu, et al. (1988), in ISF-JOCS World Conference; Yamada, et al., *J. Disp. Sci. & Tech.*, 10:561-579, 1989; and Shimizu, et al. (1989), *Appl. Microb. Biotechnol.*, 31:1-4). In these documents, it is indicated that under various fermentation conditions, *Mortierella* strains will produce cellular lipid having ARA as up to 55 % of the total lipid, with varying amounts of EPA.

Yamada, et al. (1987) reported that ARA, EPA and another highly unsaturated fatty acid, bishomo- γ -linolenic acid, were found to accumulate in the polar lipids, associated with the mycelial membrane, rather than in triglycerides (see Abstract and the sentence spanning columns 1 and 2 of page 175). Yamada, et al. (1987), described a fermentation of *M. elongata* in which the relative proportion of phospholipids and triglycerides in the lipid fraction of the resultant biomass was determined. The ARA content of the triglycerides is not disclosed, but calculations based on the data provided in this article lead to the estimate that 58% of the phospholipids was ARA while only 17% of the fatty acids in the triglyceride

fraction were ARA. By similar calculation using data in Yamada, et al.(1989), one can obtain an estimate of the ARA content in the triglyceride fraction of another fermentation. The estimated ARA content of the triglyceride fraction is about 22%, while the ARA content of the phospholipids can be estimated to be about 66%. In other words, none of the articles by the Suntory group reported triglycerides high in ARA, and where the articles provided sufficient data to allow estimation, the data indicates that none of their fermentations produced a triglyceride fraction containing at least 30% ARA.

Cited Suntory application JP 01/196,255 suggests production of infant formula which matches the fatty acid profile of human milk. This reference also teaches the manufacture of infant formula by adding either fatty acids, or oils and fats containing these fatty acids, in the appropriate ratios to produce a product having the desired amounts of the individual fatty acids. The '255 application exemplifies (Example 4) preparation of infant formula by culturing *Mortierella* microorganisms, recovering the biomass and extracting it with an organic solvent to obtain a lipid fraction as one of the ingredients in the preparation of infant formula in which the ARA/EPA ratio is 2.0. In another example, they chose to combine ethyl esters of fatty acids to produce formula having ARA/EPA ratio of 0.5. Nowhere does the '255 application specifically disclose a product with high ARA/EPA ratio, let alone the use of high ARA/low EPA oil.

Cited Japanese patent application 01/215,245 (Suntory) is directed to production of animal feed. It has no teaching regarding human food or therapeutic use. Therefore this reference has not relevance to the claims to products for human use disclosed and claimed in the present application. Furthermore, JP '245 appears to teach use of fungal biomass (i.e.,

whole microbial cells) or crude lipid extracts containing the appropriate fatty acids as ingredients in pet foods. There is no teaching of triglyceride oils according to the present invention, and products containing these triglyceride oils rather than biomass, crude lipid extracts or free fatty acids, are clearly different from the products described in the Japanese '245 application and are not suggested by JP '245.

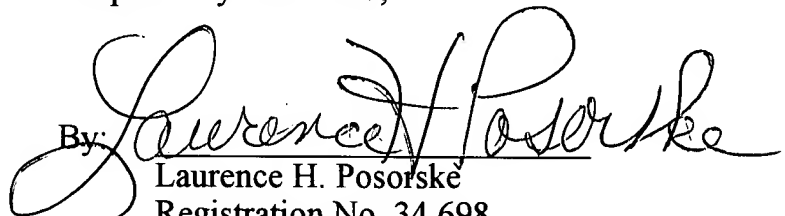
The inventors of patent applications, JP '245 and JP '255, are Shinmen, et al. In the examples of JP '255, individual fatty acid ethyl esters were combined to achieve compositions with particular ratios of fatty acids. In JP '245, fatty acids for the animal feed composition are provided as either (a) general lipid extracts, (b) fatty acid hydrolysate, (c) esters of fatty acids (see the ethyl esters disclosed above), or (d) whole fungal organisms containing the desired fatty acids. Clearly, these documents do not suggest isolation of a triglyceride fraction to obtain an oil enriched in the desired fatty acids. Thus, the prior art, and particularly the cited art, did not suggest that fungal oil would be a good source of triglycerides enriched in ARA.

Further, none of the documents by Shinmen, et al., disclose the fermentation method disclosed and claimed in the present application, requiring high substrate levels and pH profiling during fermentation of *Mortierella* to produce triglycerides having at least 25 % ARA. JP 01/304 892 (Suntory) describes producing microbes enriched in polyunsaturated fatty acids by feeding vegetable oils as carbon source, but there is no indication of what form the fatty acid residues are in. In particular, JP '892 does not describe a microbial triglyceride oil containing at least 10% ARA and substantially free of EPA.

Triglycerides containing the desired ARA residues with reduced level of EPA can be readily obtained by extraction from microbial sources produced as first taught in the present application. By this teaching, the need for further purification taught in the prior art (e.g. 9 by hydrolysis under harsh conditions followed by fractionation of individual fatty acids) is obviated. Thus, the triglyceride oil of the present invention, containing at least 30% ARA, is novel, and the prior art does not suggest methods for obtaining useful products by adding a fungal triglyceride oil having at least 10% ARA, which were first disclosed by the present inventor. Therefore, the claims of the present application are not obvious over the cited art, and Applicant respectfully requests that the rejection of the claims under 35 U.S.C. § 103 be withdrawn.

Applicant respectfully requests that the Examiner reconsider the subject application in view of the above amendments and arguments.

Respectfully submitted,

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